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Preliminary Investigation of *OLEORESIN CAPSICUM*

NIJ Report 100-95

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This report was prepared by the Office of Law Enforcement Standards (OLES) (formerly the Law Enforcement Standards Laboratory) of the National Institute of Standards and Technology (NIST) under the direction of Daniel E. Frank, Manager, Protective Equipment Program and Kathleen M. Higgins, Director of OLES.
The technical research was performed by Richard G. Christensen, Analytical Chemistry Division.
The work resulting in this report was sponsored by the National Institute of Justice, David G. Boyd, Director, Office of Science and Technology.

FOREWORD

The Office of Law Enforcement Standards (OLES) of the National Institute of Standards and Technology (formerly the National Bureau of Standards) furnishes technical support to the National Institute of Justice program to strengthen law enforcement and criminal justice in the United States. OLES's function is to conduct research that will assist law enforcement and criminal justice agencies in the selection and procurement of quality equipment.

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This document covers research conducted by OLES under the sponsorship of the National Institute of Justice. Additional reports as well as other documents are being issued under the OLES program in the areas of protective clothing and equipment, communications systems, emergency equipment, investigative aids, security systems, vehicles, weapons, and analytical techniques and standard reference materials used by the forensic community.

Technical comments and suggestions concerning this report are invited from all interested parties. They may be addressed to the Office of Law Enforcement Standards, National Institute of Standards and Technology, Gaithersburg, MD 20899.

David G. Boyd
Director, Science and Technology
National Institute of Justice

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PRELIMINARY INVESTIGATION OF OLEORESIN CAPSICUM

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This report documents a preliminary investigation into the analytical characterization of *Oleoresin Capsicum* (OC), an oily extract of hot peppers that is increasingly used in law enforcement applications. Being a natural product, it is subject to variations in composition. The characteristic hot sensation or pungency is quantified in the industry in terms of "Scoville Heat Units," (SU). SU is defined as the dilution at which the pungency can barely be detected by a trained taster. Pure capsaicin, the most pungent constituent, has an SU value of 16×10^6 mL/g (milliliters of diluent per gram of base material).

Recently, OC has come into use as a pain-producing agent in aerosol sprays used in an attempt to subdue violent individuals. This use raises the question of how to assign an objective index of potency to these sprays. Published information on the pharmacology of capsaicinoid compounds has been studied to examine correlations between pungency and pain production. Experiments in the analytical chemistry of OC have been carried out to check the feasibility of determining the concentrations of the pungent constituents.

Key words: capsaicin; incapacitation; less-than-lethal; oleoresin capsicum; pain; pepper spray; Scoville Heat Units; self-defense.

1. INTRODUCTION

This report documents a preliminary investigation into the analytical characterization of Oleoresin Capsicum (OC), an extract of hot peppers. In addition to its use in foods and pharmaceuticals, it is an active ingredient in aerosol sprays currently being used in an attempt to subdue violent individuals through inhalation and skin contact. The pungency of taste and the production of pain associated with this material are the result of a family of compounds known as capsaicinoids. The chief capsaicinoids present in OC are capsaicin (CAP), dihydrocapsaicin (DIHCAP), nordihydrocapsaicin (NORDIH), homocapsaicin (HOMO), and dihydrohomocapsaicin (DIHHOMO). Another compound which may be present is pelargonic acid vanillylamide, also called nonivamide (NONIV), a synthetic compound which has similar properties and is sometimes used with or in place of the natural compounds.

Several methods have been identified to quantify the potency of capsaicinoid preparations. For example, the taste pungency of capsaicinoid preparations is usually quantified in terms of Scoville Heat Units (SU), which is expressed as the dilution at which the preparation can barely be tasted by trained personnel [1].[1] However, the production of pain has also been quantified by one investigator in terms of a "mean pain potency" (MPP), which is the concentration required to elicit a defined pain response when the solution is applied to the eye of a rat. Another possible measure of potency, is the simple sum of the concentrations of the various capsaicinoid compounds which are present.

2. PHARMACOLOGY OF CAPSAICINOID COMPOUNDS

In the evaluation of the incapacitating effects of pepper-spray devices which project an aerosol of Oleoresin Capsicum, the question arises as to how to objectively assign some quantitative potency to given preparations. Chromatographic analysis can provide the relative concentrations of the various species of capsaicinoids that are present, so some calculations from these results might be used to assign an index which could be used as a quantitative measure of potency.

[1] Numbers in brackets refer to references in section 8.

Pharmacological effects of the topical application of this class of compounds to skin and mucous membranes include the production of pain and subsequent desensitization to pain. This effect arises from the binding of specific parts of the molecule to receptors in nerve endings [2, 3]. This pain production appears to be a temporary phenomenon only, and is not the result of tissue damage that might be produced by a corrosive agent, e.g., by a strong acid.

The pungency of capsaicinoid compounds and preparations containing them is widely quantified in terms of "Scoville Heat Units" (SU). The SU value is the dilution at which the pungency can just be detected by a trained taster. For example, pure capsaicin is assigned an SU value of 16×10^6 mL/g, implying that it can be barely detected at a dilution of sixteen million to one [1]. If the concentrations of the capsaicinoid compounds in a sample can be determined, the pungency for each can be taken into account, and an SU can be assigned to a preparation in this way.

Since the production of pain and the sensation of pungency arise through similar physiological pathways, i.e., interaction with nerve receptors, it might be supposed that the effects would be proportional. Govindarajan and Sathyanaranaya [2] tabulate data on a number of these compounds showing SU values and a pain production parameter that indicate otherwise.

Some data on capsaicinoids are given in condensed form as Table 1. The "mean pain potency" (MPP) is the concentration required to produce a certain response when a solution is applied to the eye of a rat. As such, it is an inverse indicator, that is, the smaller number implies greater potency. Note that the major capsaicinoids and nonivamide have about the same MPP. This suggests that the sum of the concentrations of these compounds will be a better (though not greatly different) index of potency than the commonly used one of SU.

It is possible to calculate from analytical data three possible measures of pungency: (a) the total concentration of capsaicinoid compounds; (b) the SU pungency (by weighting the concentration of each constituent with its relative pungency); and, (c) a mean pain potential (by weighting the concentration of each constituent according to the "MPP" of reference 2). Incapacitation will also, of course, vary with applied dosage and of the responses of individual subjects, but those are beyond the scope of this study.

TABLE 1. *Pungency and pain-producing potency of capsaicin and analogs (adapted from Refs. [1] and [3])*

Stimulant	*SU (mL/g)*	*MPP (μg/mL)*
Capsaicin	16×10^6	2.5
Dihydrocapsaicin	16×10^6	3.5
Nordihydrocapsaicin	9×10^6	3.0
Nonivamide	---[2]	2.5
Homocapsaicin	6.9×10^6	---
Dihydrohomocapsaicin	8×10^6	---

Since the purpose of this investigation was to determine how one can arrive at an objective measure of the constituents of concentrated OC, analytical determinations were carried out on five samples collected by a third party and transmitted through the Office of Law Enforcement Standards so that the source of the samples would be unknown to the researchers. They were labeled OC-1, OC-2, OC-3, OC-4, and OC-5. They all were dark red liquids of moderate viscosity, except for OC-1, which was a clear liquid of water-like consistency.

3. ANALYTICAL PROCEDURES

Liquid chromatography (LC) offers adequate separating power for this analysis. Reversed-phase LC using water/methanol/acetic acid as the mobile phase on a C-18 column was used. Oily components were removed from the samples with a solid-phase extraction clean-up step.

Achieving adequate sensitivity poses no problem in this analysis as the analytes are present at relatively high concentrations. Thermospray mass spectrometric detection (LC/MS)[3] is feasible because the compounds give a good yield of ions by protonation of the amide nitrogen. Optical absorbance detection in the ultraviolet region (LC/UV) also may be used as the vanillyl chromophore has a strong absorbance at 280 nm.

[2] --- indicates that the data were not available from the stated references.

[3] LC/MS means Liquid Chromatography followed by Mass Spectrometric detection while LC/UV means Liquid Chromatography followed by Ultraviolet absorption detection.

The LC/MS investigations were undertaken first to identify the chromatographic peaks associated with the compounds of interest based on the molecular weight of each compound. Five capsaicinoid compounds, CAP, NORDIH, DIHCAP, HOMO, and DIHHOMO, were identified in samples OC-2 to OC-5. Sample OC-1 gave one large chromatographic peak with m/z = 294.[4] This is the same m/z as is expected for NORDIH, but the chromatographic retention time is different. It is probable that this sample is a solution of NONIV and an unknown solvent. While the identity of this compound has not been verified, it is referred to in this report as NONIV.

4. ANALYSIS OF OLEORESIN CAPSICUM BY LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION

This analysis was undertaken to identify the peaks in the liquid chromatograms that are associated with capsaicinoid compounds. The following compounds, Table 2, are most likely to appear in these samples [5].

TABLE 2. *Compounds associated with oleoresin capsicum*

Compound	*Molecular weight*
Nordihydrocapsaicin	293
Capsaicin	305
Dihydrocapsaicin	307
Homocapsaicin	319
Dihydrohomocapsaicin	321

A previous report of a similar LC/MS analysis of OC compounds by Games, van der Greef, et al. [6] showed similar results, but did not make use of the thermospray interface as it was just becoming commercially available at that time.

The chromatographic separation conditions used were as follows:

flow rate = 1.0 mL/min.

solvent = 700 mL methanol + 300 mL water + 10 mL acetic acid Vydac C-18 column Type 201TP54 (The Separations Group, Hesperia CA).[5]

The column used is of the C-18 "polymeric" type. A C-18 column of "monomeric" type separated the naturally-occurring capsaicinoids well, but did not resolve the compound in OC-1 from capsaicin. Thermospray ionization was used, i.e., no discharge or electron ionization was employed. This is expected to give protonated molecular ions of this homologous series of compounds, the proton presumably attaching to the amide nitrogen. Thus, the ions are of the form $(M+H)^+$.

This mass spectrometric system does not give the structural information obtained from fragmentation caused by electron ionization, but it has proven to be very good for selective detection of known compounds.

Injections were made under selected-ion-monitoring conditions for ions at m/z = 294, 306, 308, 320, and 322. A typical set of mass chromatograms for sample OC-2 is shown in Figures 1–5. Injection point (t = 0) is at the left edge of the trace. The results for samples OC-2 through OC-5 are qualitatively similar. Good quantitative estimates of the amounts of each compound require the availability of suitable reference compounds and internal standards.

[4] m/z is the mass to charge ratio.

[5] Certain commercial products are identified in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement of the particular product by the National Institute of Standards and Technology, nor does it imply that the product identified is necessarily the best available for the purpose.

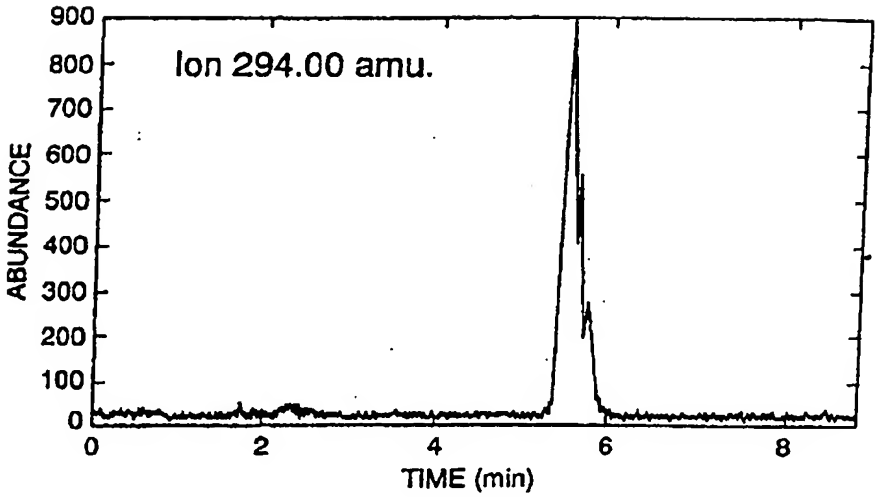

FIGURE 1. *Mass chromatogram of sample OC-2 at m/z = 294.*

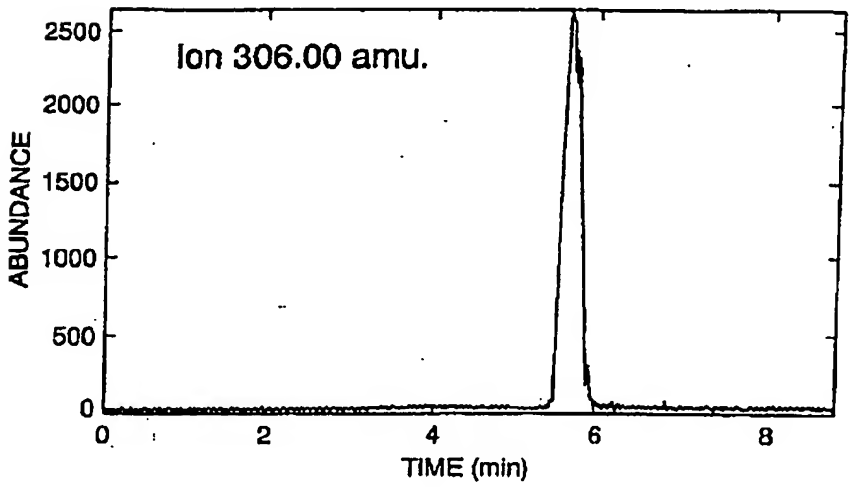

FIGURE 2. *Mass chromatogram of sample OC-2 at m/z = 306.*

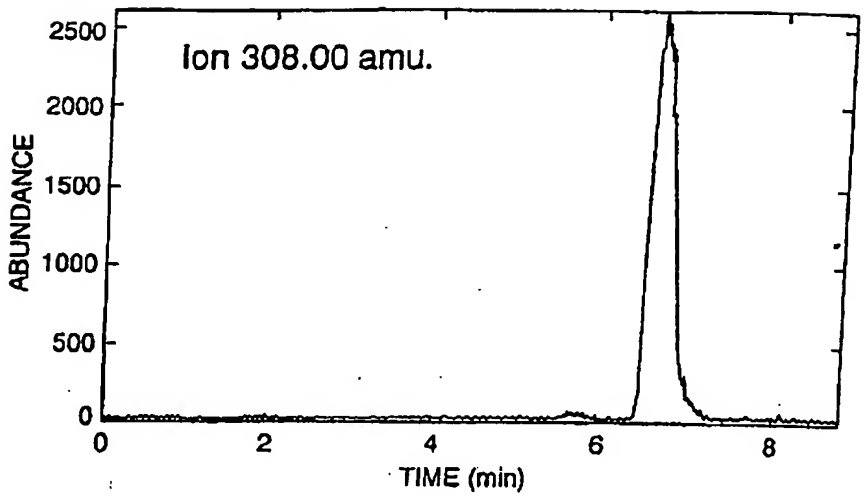

FIGURE 3. *Mass chromatogram of sample OC-2 at m/z = 308.*

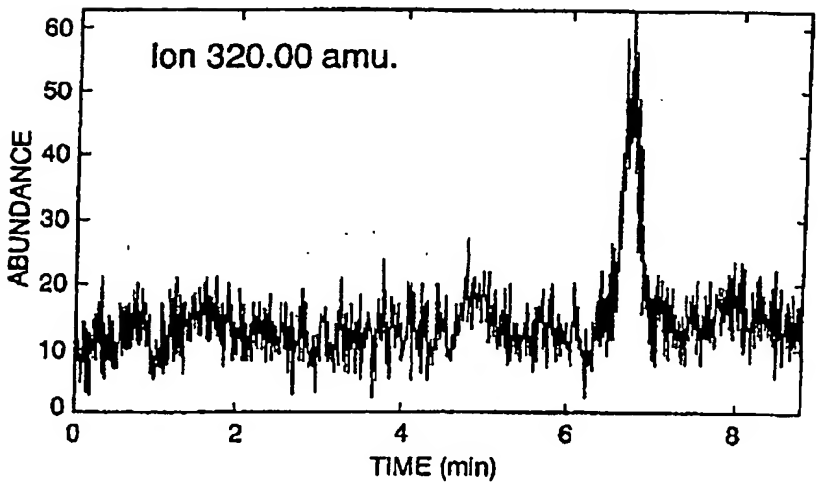

FIGURE 4. *Mass chromatogram of sample OC-2 at m/z = 320.*

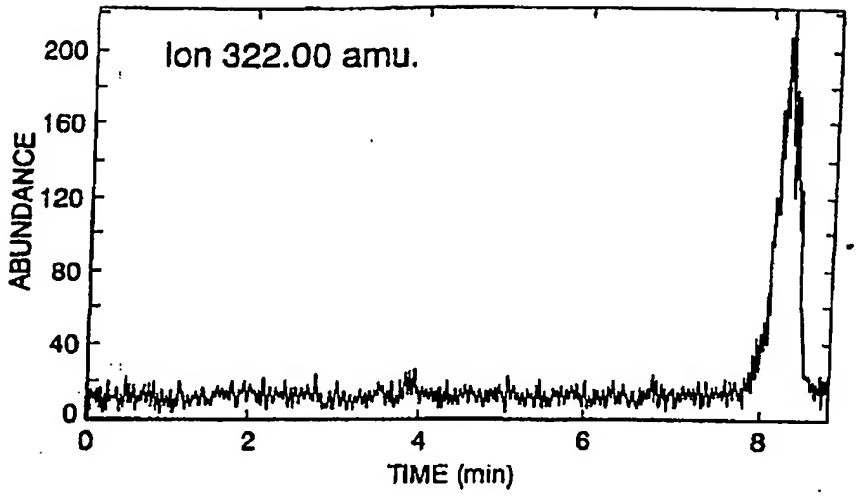

FIGURE 5. *Mass chromatogram of sample OC-2 at m/z = 322.*

The sample OC-1 showed an ion at m/z = 294, but its elution volume was different from that of nordihydrocapsaicin. In the absence of any other information, it is surmised that this sample is a solution of nonivamide, which has been used with or in place of the natural compounds, and is, in fact, known as "synthetic capsaicin" [4]. The mass chromatogram is shown as Figure 6.

Having identified the chromatographic peaks by LC/MS, LC/UV was used for quantitative measurements. Due to the short time frame for this study, the acquisition of a complete set of reference compounds was not undertaken. Therefore, only CAP was available as a pure compound to use as a standard, so the quantification of the other capsaicinoids is based on the assumption that their molar absorptivities are the same as that of CAP.

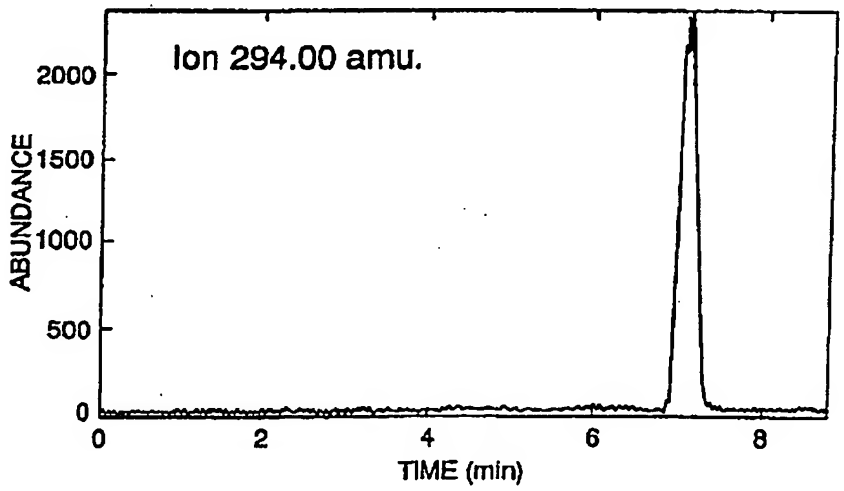

FIGURE 6. *Mass chromatogram of sample OC-1 at m/z = 294.*

5. ANALYSIS OF OLEORESIN CAPSICUM BY LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET ABSORPTION DETECTION

The LC/MS method used in this project is highly sensitive and specific for these compounds, but employs an instrument that is not widely available in analytical laboratories. The LC/UV method described here is adequate for the purpose of quantifying capsaicinoid compounds in OC preparations and uses equipment likely to be found in most analytical laboratories.

A similar chromatographic separation to that described in the section on mass spectrometric detection was used except that the flow rate was increased to 1.5 mL/min and a slightly weaker solvent, i.e., 60% methanol, was used. Capsaicin has UV absorbance maxima at 227 nm and 281 nm. The molar absorptivity at the first wavelength is about three times higher than that at the second wavelength, but since sensitivity is not a problem, the latter wavelength was chosen to minimize the risk of interferences.

Samples were also subjected to treatment with a solid-phase extraction cartridge before injection to remove possible interfering compounds. First, 100 mg of oleoresin was shaken with 2 mL acetonitrile and 2 mL of water was added. This yielded a suspension which was passed through a C-18 Sep-Pak cartridge (Waters Assoc., Milford MA). The cartridge had been conditioned with 5 mL of methanol followed by 5 mL of water. The capsaicinoid compounds were then eluted with 4 mL of a solvent composed of 80% methanol, 20% water. This sample preparation is similar in concept to the clean-up of pepper extracts described by Attuquayefio and Buckle [5].

From the solubility behavior of the samples, it is surmised that samples OC-4 and OC-5 were prepared by a different process than samples OC-2 and OC-3. The former two seemed to be more oily in consistency.

Chromatograms of each sample, with peaks labeled, are shown in Figures 7–11, corresponding to sample numbers OC-1 to OC-5, respectively. Figure 12 shows a chromatogram of a mixture of samples OC-1 and OC-3.

Comparing the sample peak areas with that of a peak obtained from injection of a solution of capsaicin yields the values for the concentration of the various capsaicinoids, assuming that the molar absorptivity at 280 nm is the same for all the compounds. The results are tabulated in Table 3.

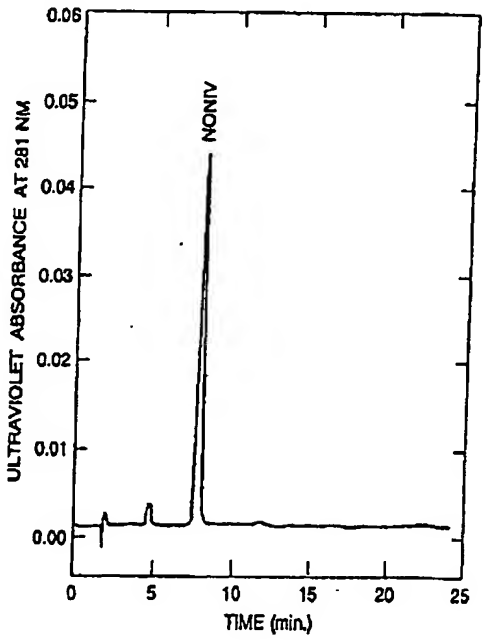

FIGURE 7. *LC/UV chromatogram of OC-1.*

[6] See NIST Technical Note 1297, Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results, for further information.

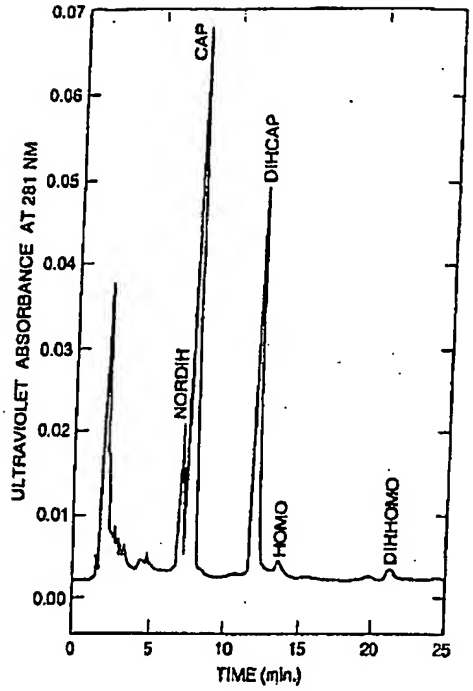

FIGURE 8. *LC/UV chromatogram of OC-2.*

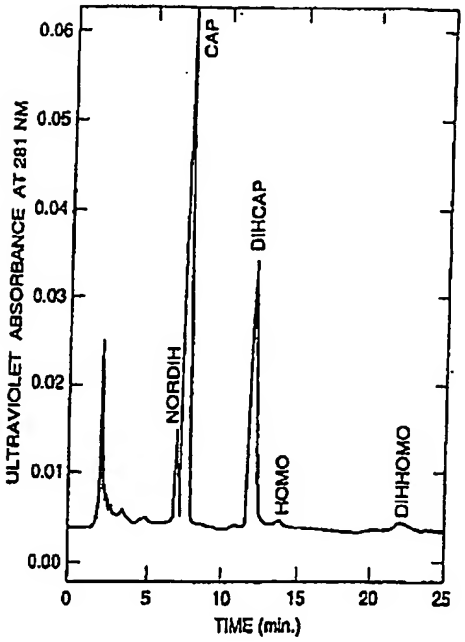

FIGURE 9. *LC/UV chromatogram of OC-3.*

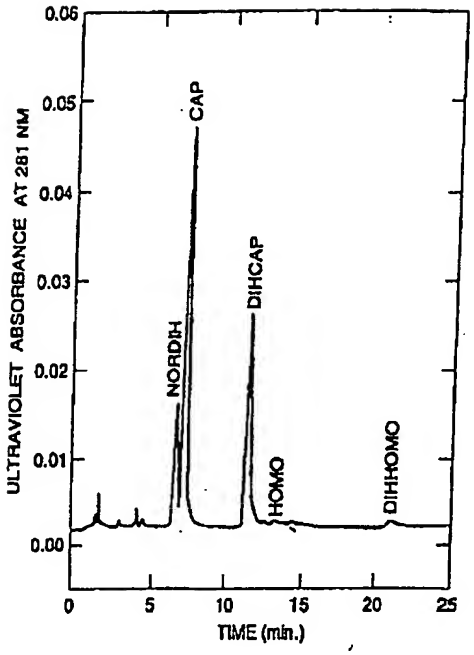

FIGURE 10. *LC/UV chromatogram of OC-4.*

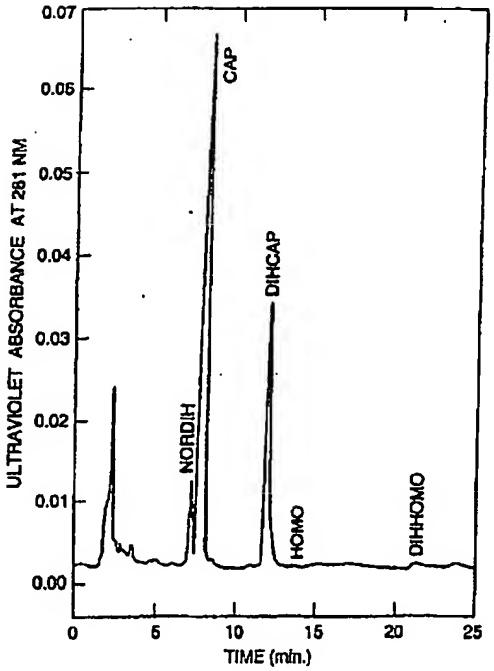

FIGURE 11. *LC/UV chromatogram of OC-5.*

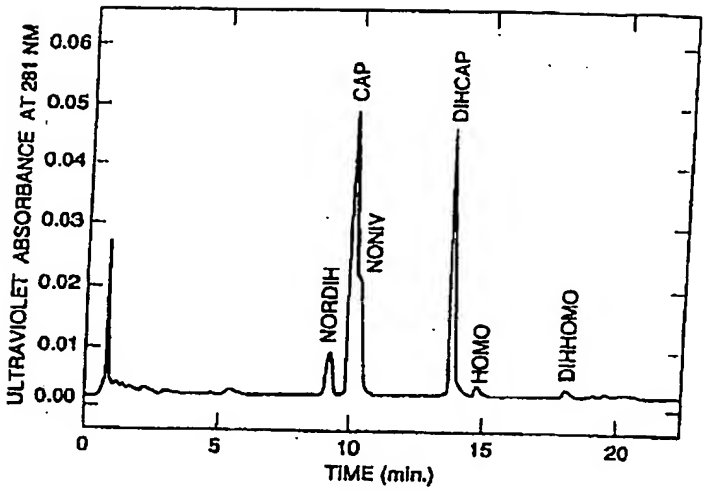

FIGURE 12. *LC/UV chromatogram of OC-3 + OC-1.*

TABLE 3. *Capsaicinoid concentrations in samples OC-1 through OC-5 (in Mg/g)*

Compound	*OC-1*	*OC-2*	*OC-3*	*OC-4*	*OC-5*
NORDIH	---	11	8	2.8	3.1
CAP	---	56	53	13	27
DIHCAP	---	55	42	10	29
HOMO	---	1.7	1.7	---	---
DIHHOMO	---	2.8	2.4	---	---

The blank (---) spaces indicate that the compound was not detected or was present at a very low concentration. Replicate determinations were not made. This type of analysis usually yields results that have relative expanded standard uncertainties of 5%–10% (coverage factor = 2. This approximates a 95% confidence level).[6] The chromatographic separation is highly repeatable, but the robustness of the method with respect to the solid-phase extraction sample clean-up is not known. OC is a natural product and is presumably prepared by various extraction processes. Different starting materials or different manufacturing processes may give rise to measurement biases owing to variations in the matrix. Further experience with the analysis would be necessary to rule out or to overcome problems from this source.

6. RESULTS

From the determination of the concentrations of capsaicinoids in the several samples, three types of figures of merit for potency have been calculated. The first is the simple sum of the concentrations of all the capsaicinoids found. The second is the sum of the Scoville Heat Unit values of the constituents. It was calculated by summing the products of the concentrations and the SU values published for each compound. The third is a "Pain Index." Since the MPP of Govindarajan is the inverse of SU (having units of μg/mL, microgram per milliliter, rather than SU's units of mL/g), it is possible to calculate a quantity analogous to SU by dividing the concentration of each constituent by its MPP. The Pain Index contributions for each constituent are then summed for each sample. Each of these three parameters for each sample are shown in Table 4.

[6] See NIST Technical Note 1297, Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results, for further information.

TABLE 4. *Possible potency indices for oleoresin capsicum*

Sample	*Capsaicinoid concentration mg/g (milligram per gram)*	*Scoville Heat Units, mL/g (milliliters per gram)*	*Pain Index mL/g (milliliters per gram)*
OC-1	12	0.11×10^5	5000
OC-2	126	1.9×10^6	42000
OC-3	107	1.6×10^6	36000
OC-4	25	0.38×10^6	9000
OC-5	61	0.94×10^6	20000

It is easily seen that independent of which measure is used the relative ranking from smallest number to highest number is OC-1, OC-4, OC-5, OC-3, and OC-2.

7. CONCLUSION

From the chromatograms, figures 7 through 12, it can be seen that it is possible to detect the presence of the possible synthetic compound, NONIV, in the presence of OC; and, that it is possible to obtain the quantitative data needed to characterize OC preparations using ordinary LC equipment. The additional specificity and sensitivity of LC/MS are useful in identifying chromatographic peaks but are not necessary for objectively determining the potency of a preparation.

As can be seen from the ranking table above, the several methods described in this report for assigning a value for the potency of a mixture of the compounds occurring in Oleoresin Capsicum tend to give results which are proportional to one another. The attraction of the first method is that it is straightforward; the drawback is that it does not take into account the variations which are known to exist between capsaicinoid compounds. The second and third methods do take these variations into account, but the SU and MPP values are based on stimulus values, which are subjective assignments.

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CAP-STUN® OC Products

Oleoresin Extraction

The industrial spice Oleoresin extraction industry came into being with the development of Oleoresin process during the 1930's. The process essentially involves concentration of the oleoresin from capsicum plant by evaporation of solvent and, finally desolventisation to achieve the limits of residual solvent. Oleoresin, being a natural product, is thermally sensitive and the processing must be designed to minimize thermal degradation and preserve the full pungency. Conventional concentration and desolventisation techniques employ batch evaporation. In this process the oleoresin gets cooked over an extended length of time, which directly diminishes the Oleoresin quality.

Oleoresin Capsicum (OC) is therefore the extract of the dried ripe fruits of Capsicums and contains a complex mixture of essential oils, waxes, colored materials, and several capsaicinoids. It also contains resin acids and their esters, terpenes, and oxidation or polymerization products of these terpenes. One kilogram of Oleoresin Capsicum is equivalent to approximately 18 to 20 kilograms of good grade well-ground capsicum. This ratio may vary depending on the type of capsicum being processed.

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Quantitative Analysis of Capsaicinoids in Fresh Peppers, Oleoresin Capsicum and Pepper Spray Products

REFERENCE: Reilly CA, Crouch DJ, Yost GS. Quantitative analysis of capsaicinoids in fresh peppers, oleoresin capsicum and pepper spray products. J Forensic Sci 2001;46(3):502–509.

ABSTRACT: Liquid chromatography-mass spectrometry was used to identify and quantify the predominant capsaicinoid analogues in extracts of fresh peppers, in oleoresin capsicum, and pepper sprays. The concentration of capsaicinoids in fresh peppers was variable. Variability was dependent upon the relative pungency of the pepper type and geographical origin of the pepper. Nonivamide was conclusively identified in the extracts of fresh peppers, despite numerous reports that nonivamide was not a natural product. In the oleoresin capsicum samples, the pungency was proportional to the total concentration of capsaicinoids and was related by a factor of approximately 15,000 Scoville Heat Units (SHU)/μg of total capsaicinoids. The principle analogues detected in oleoresin capsicum were capsaicin and dihydrocapsaicin and appeared to be the analogues primarily responsible for the pungency of the sample. The analysis of selected samples of commercially available pepper spray products also demonstrated variability in the capsaicinoid concentrations. Variability was observed among products obtained from different manufacturers as well as from different product lots from the same manufacturer. These data indicate that commercial pepper products are not standardized for capsaicinoid content even though they are classified by SHU. Variability in the capsaicinoid concentrations in oleoresin capsicum-based self-defense weapons could alter potency and ultimately jeopardize the safety and health of users and assailants.

KEYWORDS: forensic science, pepper spray, capsaicin, LC/MS

For centuries, people have used concentrated extracts of peppers (oleoresin capsicum) or the dried fruits to prepare spicy foods (1–4). Other historical and current uses of pepper products include neurobiological research (5), weight loss (6–8), local anesthesia (5,9), anti-microbial defense (10), anti-inflammation preparations (5,11), and recently for the production of self-defense and less-than-lethal (LTL) weaponry (3,12). The extensive use of peppers and pepper extracts for such diverse purposes emanates from the presence of capsaicinoids in the pepper.

The term "capsaicinoids" describes a group of pungent chemical analogues found in hot peppers (*Capsicum annum* and *C. frutescens*) (1–4). There are five naturally occurring capsaicinoids: capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin (1–4). The most abundant and potent analogues in peppers (and consequently pepper extracts) are capsaicin and dihydrocapsaicin (1–4,13). Nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin are also present, but generally contribute little to the total capsaicinoid concentration and pungency of the pepper (1–4,13). Nonivamide, or "synthetic" capsaicin, exhibits the same pungency as capsaicin, but has not been conclusively identified as a natural product (3,13). In fact, some scientists have concluded that detection of nonivamide in oleoresin capsicum was indicative of adulteration of the sample (3). The total concentration of capsaicinoids in a pepper ranges from 0.1 to 2.0% (dry weight) and depends upon the variety of the pepper, the growing conditions, and the time of harvest (1,3,4). In addition to differences in the total capsaicinoid content, variations in the relative proportions of the capsaicinoid analogues also occurs in response to the above criteria (1–4).

Capsaicinoids are the pharmacologically active and pain-producing components of the hot pepper (4). The characteristic chemical structure of capsaicin (or its analogues) contains a vanillamide moiety (4-hydroxy-3-methoxybenzylamide) and an acyl chain containing 10 to 11 carbon atoms (Fig. 1) (1–4,12). Capsaicinoids produce pain by stimulating the vanilloid receptor. This receptor is a molecular integrator of potentially noxious stimuli (e.g., low pH and high temperature) (5,11,14,15). Structure activity studies of capsaicin and other related compounds have demonstrated a strict requirement for the vanillyl ring and an acyl chain of 8 to 12 carbon atoms to manifest pungency (16–18). The natural capsaicinoids exhibit variable pungency due to differences in their ability to promote membrane depolarization through binding to the vanillamide receptor.

The ability of the capsaicinoids to produce pain has prompted the development of pepper sprays for the purpose of self-defense. Typically, pepper sprays weapons contain a 10% solution of oleoresin capsicum diluted in a suitable solvent (e.g., methylene chloride, trichloroethylene, isopropanol, freon(s), propylene glycol, ethanol, methanol, or dimethyl ether) and a gaseous propellant (usually N_2 or CO_2). Exposure to pepper sprays elicits an intense physiological response that includes nociception, temporary blindness, lacrimation, disorientation, shortness of breath, and choking (19). The result of the exposure is temporary incapacitation of the victim with minimal long-term side effects and/or toxicity (19,20).

Unfortunately, the manufacturers of oleoresin capsicum and self-defense weaponry employ few, if any, analytical measures to determine the concentration of active ingredients in the product and to ensure consistent chemical composition. Variability in the concentration of active ingredients might explain why pepper sprays have been shown to be only 70% effective in discouraging attacks by aggressive individuals (20). The principal test for product composition is a taste test to determine the product's ability to elicit

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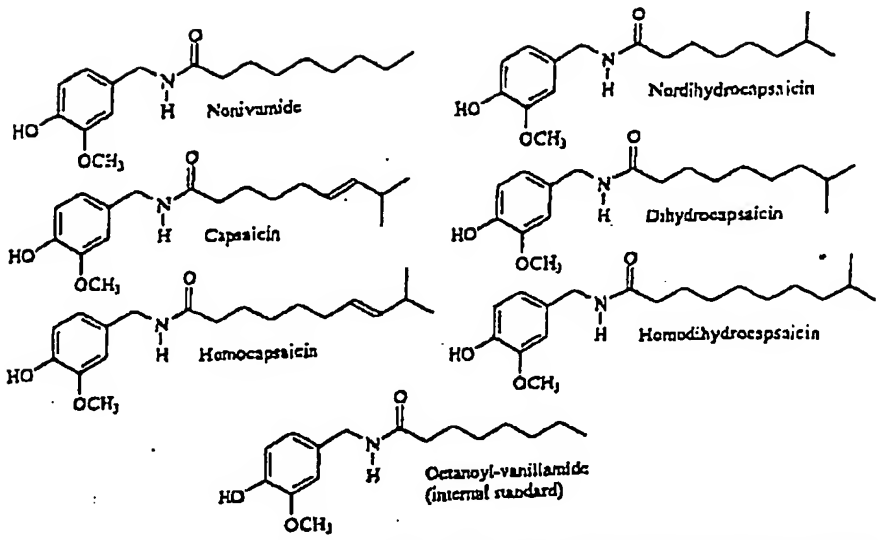

FIG. 1—*Chemical structures of the capsaicinoid analogues and octanoyl-vanillamide (internal standard).*

pain. This test is known as the Scoville Organoleptic Test (21). Unfortunately, the Scoville Organoleptic Test gives only a subjective measure of potency (SHU) rather than a quantitative assessment of the capsaicinoid concentration (21). Since the concentration of active ingredient in pepper products (including pepper sprays) is only subjectively assessed, the potency, efficacy, and potential product toxicity is difficult to predict (22).

In this study, we identified and quantified the individual capsaicinoid analogues in various fresh peppers, oleoresin capsicums, and pepper spray weapons. Nonivamide was identified in five different types of fresh peppers originating from different locations, as well as in all commercial pepper products. We also calculated the relationship between pungency and capsaicinoid concentration in oleoresin capsicum as well as in a series of pepper-based self-defense products.

Materials and Methods

Chemical

Extreme caution must be used when handling capsaicinoids and/or pepper products to prevent serious discomfort and/or injury. Capsaicin (60% purity), capsaicin (*E*-8-methyl-N-vanillyl-6-nonenamide) (98% purity), dihydrocapsaicin (8-methyl-N-vanillylnonanamide) (98% purity), and nonivamide (N-vanillylnonanamide) were purchased from Sigma Chemical Co. (St. Louis, MO). Nordihydrocapsaicin, *E*-homocapsaicin, and homodihydrocapsaicin were purified from oleoresin capsicum using HPLC. Vanillamine hydrochloride and octanoyl chloride were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Octanoyl-vanillamide was synthesized by condensation of vanillamine and octanoyl chloride as previously described (12). HPLC-grade methanol and reagent-grade *n*-butyl chloride were purchased from Burdick and Jackson (Muskegon, MI). Formic acid, sodium phosphate, and sodium chloride were purchased from Mallinckrodt (Paris, KY). The oleoresin capsicum samples were obtained from various spice vendors and the pepper sprays were purchased from independent distributors.

Analytical Methods

Identification and quantification of the individual capsaicinoids was performed by liquid chromatography-mass spectrometry using a Hewlett-Packard series 1100 LC/MSD (Agilent Technologies, Palo Alto, CA). The capsaicinoids were separated using a MetaSil Basic reversed-phase HPLC column (100 × 3.0 × 3μ) (MetaChem Technologies, Torrance, CA) and a stepwise gradient of methanol and distilled water containing 0.1% (v/v) formic acid. The mass spectrometer was set to detect the M+H positive ions of octanoyl-vanillamide (m/z = 280), nonivamide (m/z = 294), nordihydrocapsaicin (m/z = 294), capsaicin (m/z = 306), dihydrocapsaicin (m/z = 308), homocapsaicin (m/z = 320), and homodihydrocapsaicin (m/z = 322).

Preparation of the analytical standards was achieved by weighing the appropriate quantity of each analogue on a Cahn 4700 analytical balance (Cahn Instruments, Cerritos, CA) and dissolving the compounds in methanol. Standards were stored at −20°C and were stable for the duration of the study. Calibration curves, containing all analogues and 500 ng octanoyl-vanillamide (as an internal standard), were constructed from 0 to 5000 ng. Calibration curves were generated by calculating the peak area ratio (obtained using HP Chemstation software) of the analyte to internal standard. Linear regression analyses were performed using the least squares method. Separate curves were used to quantify the low- (1 to 50 ng), mid- (50 to 500 ng), and high- (500 to 5000 ng) range concentrations of the analogues. Split curves were needed to more accurately quantify the concentration of the compounds in the samples.

Analysis of Fresh Peppers

Extracts of fresh peppers were prepared by homogenizing approximately 5 to 50 g of fresh pepper (purchased from food stores in UT, NM, and MD) in 5 volumes of phosphate-buffered saline (50 mM phosphate buffer, pH 7.2 containing 1 *M* NaCl) using a Teflon/glass homogenizer. To decrease the sample size of the fresh peppers, the stem and a portion of the pericarp were removed; the stem and pericarp have been shown to contain only negligible con-

centrations of the capsaicinoids (1,23,24). The homogenate was extracted for 15 min at room temperature with *n*-butyl chloride. The extract was centrifuged at 1000 × g for 10 min and the upper organic layer was collected. The extraction process was repeated and the organic layers were combined and evaporated to dryness under a stream of air at 40°C. The dried residues were reconstituted in 200 µL *n*-butyl chloride:methanol (1:1). The reconstituted residues were diluted 20-fold in *n*-butyl chloride:methanol (1:1) and a 5 µL aliquot was combined with 500 ng octanoyl-vanillamide. The samples were evaporated to dryness, reconstituted with 100 µL of 70% methanol: 30% distilled H_2O, and analyzed using LC/MS.

Analysis of Oleoresin Capsicum

Commercially available oleoresin capsicum samples were diluted 200-fold in *n*-butyl chloride:methanol (1:1). A 5 µL aliquot of the diluted product was pipetted into a 13 × 100 mm silanized glass tube and octanoyl-vanillamide (500 ng) was added as the internal standard. The sample was evaporated to dryness, reconstituted with 100 µL of 70% methanol: 30% distilled H_2O, and analyzed using LC/MS.

Analysis of Pepper Spray

Individual pepper spray canisters were vigorously shaken and cooled to −20°C overnight in a freezer. The sprays were then gently discharged into a 16 × 100 silanized glass tube that had been previously equilibrated to −80°C using dry ice. Cooling the tubes with dry ice was necessary to prevent evaporation of the solvents during collection. The sample was immediately capped and thawed on ice. The sample volume was determined and the volatile components were permitted to evaporate at room temperature with gentle agitation. The sample volume was reestablished by adding *n*-butyl chloride:methanol (1:1). Samples were then diluted 40-fold in *n*-butyl chloride:methanol (1:1) and a 5 µL aliquot combined with octanoyl-vanillamide (500 ng). The samples were evaporated to dryness, reconstituted with 100 µL of 70% methanol: 30% dH_2O, and analyzed using LC/MS.

Results

Analysis of Capsaicinoids by LC/MS

Identification of the individual capsaicinoid analogues (see Fig. 1) was achieved by LC/MS. The calibration curve for the assay was linear from 1.0 to 5000 ng, but was split into three curves to more accurately calculate the concentration of the analytes in the samples. Identification of the individual capsaicinoids was achieved by mass-selective detection as well as by retention time. Octanoyl-vanillamide (*m/z* = 280) eluted at approximately 8.4 min followed by nordihydrocapsaicin (*m/z* = 294; 11.8 min), nonivamide (*m/z* = 294; ~12.6 min), capsaicin (*m/z* = 306; ~12.6 min), dihydrocapsaicin (*m/z* = 308; ~15.8 min), homocapsaicin (*m/z* = 320; ~16.1 min), and homodihydrocapsaicin (*m/z* = 322; ~17.5 min). A typical mass-chromatogram of a standard containing 500 ng of all analytes and internal standard is shown in Fig. 2. The compound

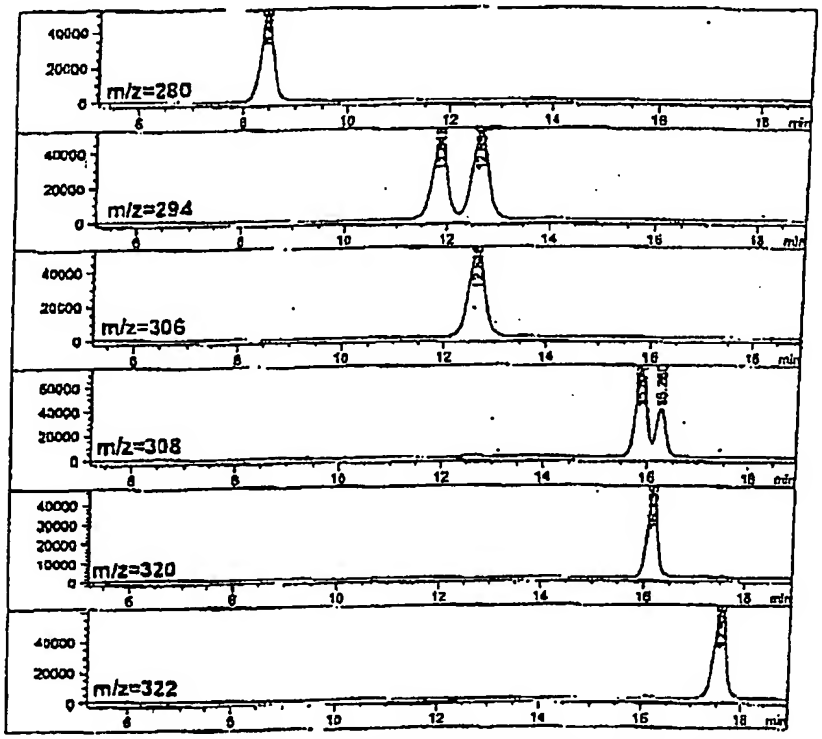

FIG. 2—*Representative reconstructed ion mass-chromatogram of a 500 ng standard mixture containing all capsaicinoids and internal standard (500 ng). Octanoyl-vanillamide is represented by the panel labeled m/z = 280, nordihydrocapsaicin and nonivamide by m/z = 294, capsaicin by m/z = 306, dihydrocapsaicin by m/z = 308, homocapsaicin by m/z = 320, and homodihydrocapsaicin by m/z = 322. Specific retention times for the individual analytes are presented in the results section. Data were collected as described under materials and methods.*

(m/z = 308) eluting at approximately 16.2 min has been tentatively identified as decanoyl-vanillamide, based on similarities in retention time and mass when compared to a synthetic standard of decanoyl-vanillamide (data not shown).

Analysis of Capsaicinoids in Fresh Peppers

The analysis of "unadulterated" pepper extracts demonstrated the presence of all previously documented naturally occurring capsaicinoids as well as nonivamide. The identification of nonivamide in peppers was confirmed by mass (m/z = 294) and retention time (approximately 12.6 min). Confirmation of the presence of nonivamide in the peppers was achieved using collision-induced fragmentation of the molecular ion by LC/MS/MS and obtaining the product ion spectrum (data not shown). Nonivamide was present in habañero, anaheim, red-chili, green-chili, and green bell pepper extracts at a relative concentration range of 0.42 to 7.2% of the total capsaicinoid concentration, depending upon the variety of pepper. Nonivamide was not detected in extracts of yellow bell pepper and was present in the green bell pepper at less than 0.025% of the concentration in green-chili pepper. The concentrations of the capsaicinoid analogues in the six varieties of peppers are summarized in Table 1.

Analysis of Oleoresin Capsicum

As described, quantification of the individual capsaicinoid analogues in commercial preparations of oleoresin capsicum was achieved by comparing the peak area ratio of the samples to the calibration curves. The capsaicinoid analogue concentrations in seven different oleoresin capsicum samples are summarized in Table 2. The absolute and relative concentrations of the individual capsaicinoids varied between samples. Figures 3A and 3B illustrate the correlation between SHU rating and the total (Fig. 3A) and individual capsaicinoid analogue concentrations (Fig. 3B). The relationship between total capsaicinoid concentration and SHU rating was approximately 15 000 SHU/μg of total capsaicinoids. Data presented in Fig. 3B indicate that capsaicin and dihydrocapsaicin were primarily responsible for the SHU rating. Their relationships were approximately 35 700 and 33 900 SHU/μg, respectively. The total and relative analogue concentrations were consistent between oleoresin capsicum samples having the same SHU rating.

TABLE 1—*Capsaicinoid analogue concentrations in extracts of fresh peppers.*

Variety of Pepper	Total (μg/g)*	NDHC† (%)	Nonivamide (%)	Capsaicin (%)	DHC† (%)	HC† (%)	HDHC† (%)
Yellow Bell (Utah)	0.0018 ± 0.0002	N.D.	N.D.	37 ± 1.0	63 ± 5.0	N.D.	N.D.
Green Bell (Utah)	0.0049 ± 0.0004	13.1 ± 0.9	7.2 ± 0.4	27 ± 2.0	52 ± 7.0	N.D.	N.D.
Green-chili (Utah)	19 ± 2.0	22.4 ± 0.2	2.7 ± 0.2	21 ± 6.0	46 ± 2.0	0.9 ± 0.4	7 ± 2.0
Anaheim (New Mexico)	73 ± 7.0	11.8 ± 0.9	1.0 ± 0.1	47 ± 2.0	36 ± 3.0	1.3 ± 0.4	3.0 ± 0.7
Anaheim (Maryland)	87 ± 5.0	6.3 ± 0.8	0.42 ± 0.07	60 ± 9.0	30 ± 4.0	2.6 ± 0.5	1.4 ± 0.3
Red-Chili (New Mexico)	83 ± 9.0	11 ± 1.0	0.6 ± 0.1	56 ± 4.0	29 ± 2.0	1.0 ± 0.5	1.8 ± 0.4
Red-chili (Utah)	59 ± 6.0	9.6 ± 0.4	1.2 ± 0.1	50 ± 3.0	27 ± 1.0	6.1 ± 0.4	6.2 ± 0.5
Habañero (Utah)	510 ± 27.0	3.6 ± 0.3	1.4 ± 0.1	61 ± 6.0	32 ± 3.0	0.9 ± 0.1	1.1 ± 0.1

* Approximately 5 to 50 g of fresh pepper was extracted as described under materials and methods. Data are representative of the mean ± standard deviation of triplicate analysis of pepper extracts.
† Abbreviations for Nordihydrocapsaicin (NDHC), Dihydrocapsaicin (DHC), Homocapsaicin (HC), and Homodihydrocapsaicin (HDHC).

TABLE 2—*Capsaicinoid analogue concentrations for various oleoresin capsicum samples.*

Sample Identity‡	Total (μg/μL)*	NDHC† (%)	Nonivamide (%)	Capsaicin (%)	DHC† (%)	HC† (%)	HDHC† (%)
OC1	0.8 ± 0.2	16.7 ± 0.3	1.8 ± 0.4	38 ± 1.0	36 ± 2.0	7.0 ± 3.0	N.D.
OC2	14 ± 1.0	20 ± 2.0	1.9 ± 0.2	36 ± 4.0	38 ± 3.0	2.0 ± 0.1	1.9 ± 0.2
OC3	18.4 ± 0.4	18.7 ± 0.3	1.2 ± 0.9	35.9 ± 0.5	39.3 ± 0.4	2.0 ± 0.1	2.9 ± 0.4
OC4	63 ± 1.0	11 ± 1.0	3.4 ± 0.5	33 ± 2.0	48 ± 3.0	1.4 ± 0.2	2.9 ± 0.7
OC5	71 ± 2.0	8.6 ± 0.7	5.2 ± 0.8	39 ± 3.0	42 ± 1.0	1.5 ± 0.1	2.4 ± 0.3
OC6	67 ± 3.0	6.3 ± 0.4	5.5 ± 0.2	47.8 ± 0.9	35.8 ± 0.7	1.7 ± 0.8	2.1 ± 0.1
OC7	131 ± 5.0	7.7 ± 0.1	4.2 ± 0.1	41.2 ± 0.5	43.5 ± 0.9	1.3 ± 0.2	2.2 ± 0.4

* Data are representative of the mean ± standard deviation of triplicate analysis of the oleoresin capsicum sample.
† Abbreviations for Nordihydrocapsaicin (NDHC), Dihydrocapsaicin (DHC), Homocapsaicin (HC), and Homodihydrocapsaicin (HDHC).
‡ Represents oleoresin capsicum samples with the following SHU ratings: OC1 (2.0×10^4 SHU), OC2 (2.0×10^5 SHU), OC3 (5.0×10^5 SHU), OC4-6 (1.0×10^6 SHU), OC7 (2.0×10^6 SHU). Although OC4-6 have the same SHU rating, they were obtained from different manufacturers of oleoresin capsicum.

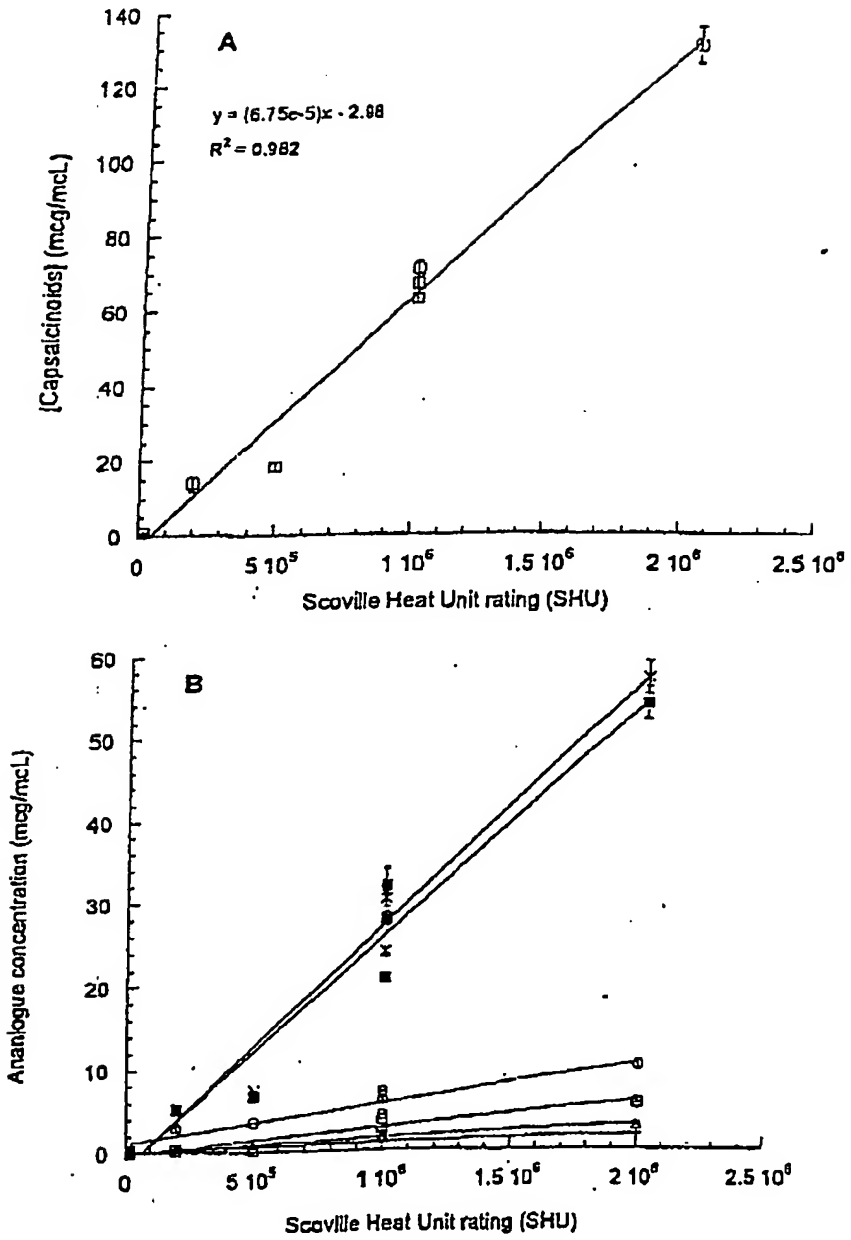

FIG. 3—(A) The relationship between Scoville Heat Unit rating and total capsaicinoid concentration in oleoresin capsicum. (B) The relationship between Scoville Heat Unit rating and the concentration of individual capsaicinoid analogues in oleoresin capsicum: (○) nordihydrocapsaicin ($y = 4.7 \times 10^{-6} x + 1.0$), (□) nonivamide ($y = 3.17 \times 10^{-6} x - 0.3$), (△) homodihydrocapsaicin ($y = 1.5 \times 10^{-6} x - 0.02$), (■) capsaicin ($y = 2.8 \times 10^{-5} x - 1.9$), (×) dihydrocapsaicin ($y = 2.95 \times 10^{-5} x + 2.0$), and (+) homocapsaicin ($y = 8.5 \times 10^{-7} x + 0.1$). Data represent the mean ± standard deviation of triplicate samples. Analysis of the capsaicinoids in oleoresin capsicum was performed as described under materials and methods.

Analysis of Capsaicinoids in Pepper Spray

Total and individual capsaicinoid analogue concentrations for several commercially available pepper spray products are summarized in Table 3. The total capsaicinoid concentrations were consistent with previous reports of the composition of pepper sprays (25). The capsaicinoid concentrations in pepper spray products from different product lots from the same manufacturer are also presented in Table 3. A comparison between the labeled and calculated SHU values for the pepper sprays is shown in Table 4. Collectively, these data demonstrate that there were substantial differences in the total and relative capsaicinoid concentrations in the pepper spray products. Also, the labeled SHU values of the pepper sprays were not consistent with the assayed values.

TABLE 3—*Capsaicinoid analogue concentrations of various pepper sprays.*

Sample Identity	Total (μg/μL)*	NDHC† (%)	Nonivamide (%)	Capsaicin (%)	DHC† (%)	HC† (%)	HDHC† (%)
PS1‡	16.0 ± 0.4	6.4 ± 0.2	1.6 ± 0.1	51 ± 1.0	37.1 ± 0.3	2.2 ± 0.1	1.5 ± 0.1
PS2‡	11.8 ± 0.4	8.7 ± 0.3	2.0 ± 0.1	45 ± 2.0	40 ± 1.0	2.0 ± 0.1	2.1 ± 0.1
PS3	0.95 ± 0.03	5.7 ± 0.2	1.8 ± 0.1	52.4 ± 0.9	37 ± 1.0	2.4 ± 0.3	0.8 ± 0.1
PS4	8.3 ± 0.4	0.2 ± 0.01	1.9 ± 0.1	50 ± 3.0	35 ± 1.0	1.6 ± 0.1	2.0 ± 1.0
PS5§	2.5 ± 0.2	9.7 ± 0.6	1.9 ± 0.1	41 ± 3.0	41 ± 3.0	2.2 ± 0.2	2.5 ± 0.9
PS6§	13.5 ± 0.1	10.8 ± 0.1	1.8 ± 0.1	36.5 ± 0.2	45.3 ± 0.3	1.8 ± 0.1	2.0 ± 0.8
PS7	13 ± 1.0	9.6 ± 0.9	2.2 ± 0.2	42 ± 5.0	42 ± 4.0	1.8 ± 0.2	1.9 ± 0.8
PS8‖	27 ± 3.0	N.D.	100 ± 10.0	N.D.	N.D.	N.D.	N.D.
PS9‖	32 ± 1.0	N.D.	100 ± 4.0	N.D.	N.D.	N.D.	N.D.

* Total capsaicinoid concentrations (μg/μL) of dispensed pepper spray. This value does not represent the total capsaicinoid content in the canister. Data are representative of the mean ± standard deviation of triplicate analysis of the pepper spray product.
† Abbreviations for Nordihydrocapsaicin (NDHC), Dihydrocapsaicin (DHC), Homocapsaicin (HC), and Homodihydrocapsaicin (HDHC).
‡, §, ‖ Represents pepper sprays from the same manufacturer, but different product lots.

TABLE 4—*Calculated versus labeled SHU value for various pepper spray products.*

Sample Identity	Total (μg/μL)*	Labeled SHU	Calculated SHU
PS1	16.0 ± 0.4	2.0×10^6	2.4×10^5
PS2	11.8 ± 0.4	2.0×10^6	1.8×10^5
PS3	0.95 ± 0.03	2.0×10^6	1.5×10^4
PS4	8.3 ± 0.4	2.0×10^6	1.3×10^5
PS5	2.5 ± 0.2	1.5×10^6	3.7×10^4
PS6	13.5 ± 0.1	1.5×10^6	2.0×10^5
PS7	13 ± 1.0	1.5×10^6	1.9×10^5
PS8†	27 ± 3.0	1.0×10^6	9.0×10^5
PS9†	32 ± 1.0	1.0×10^6	1.0×10^6

* Data are representative of the mean ± standard deviation of triplicate analysis of the pepper spray products.
† Calculated SHU values were determined using the formula SHU = [capsaicin] (μg/μL) × 33 000 (SHU/μg) since only nonivamide was present (nonivamide has similar pungency to capsaicin (13) and the concentration was not in range for the trend line for nonivamide).

Discussion

The data demonstrate that capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, and nonivamide can be found in fresh peppers, oleoresin capsicum, and pepper spray products. However, the total and relative concentrations of the capsaicinoid analogues in the peppers and the pepper products were quite variable (Tables 1 to 3). In all samples, capsaicin and dihydrocapsaicin constituted 60 to 90% of the total capsaicinoid concentration, followed by nordihydrocapsaicin (2 to 20%), homocapsaicin (1 to 5%), homodihydrocapsaicin (1 to 5%), and nonivamide (1 to 5%).

Capsaicin, nonivamide, and dihydrocapsaicin are the most pungent capsaicinoid analogues having 100, 100, and 75% relative pungencies, respectively (12,13,18). Nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin exhibit relative pungencies that range between 20 to 50% that of capsaicin (12,13,18). Because nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin contribute little to the relative pungency of the peppers or pepper products, their contribution to pungency is not discussed below. However, this does not imply that these analogues may not be important in the toxicological and/or pharmacological properties of the products.

To determine the source of variability in pepper sprays and oleoresin capsicum, we first analyzed the capsaicinoid concentrations in fresh peppers. In the extracts of fresh peppers, the total concentration of the capsaicinoids, as well as that of each analogue, was variable and dependent upon the variety and geographical origin of the pepper. The total capsaicinoid concentration in extracts of the fresh peppers was reflective of the relative "hotness" of the pepper (habeñero>anaheim = red-chili>green-chili>green bell>yellow bell). Capsaicin and dihydrocapsaicin were the most abundant capsaicinoid analogues present in the extracts of fresh peppers and comprised 60 to 90% of the total capsaicinoid concentration. The sum of the concentrations of capsaicin and dihydrocapsaicin in the extracts of fresh pepper paralleled the relative pungency of the pepper type and appeared to dictate the pungency of the fruit (see Table 1). For example, extracts of habeñero peppers (the "hottest" pepper) contained approximately 90% capsaicin and dihydrocapsaicin while extracts of the milder green-chili pepper contained about 65% capsaicin and dihydrocapsaicin.

The immediate precursor to pepper sprays is oleoresin capsicum, the extracted product of hot peppers. As expected, we also observed variability in the total and relative concentrations of the capsaicinoids in commercial preparations of oleoresin capsicum. We found that there was a strong relationship between the concentrations of the capsaicinoids in commercial preparations of oleoresin capsicum and the SHU rating of the product (Fig. 3A). Comparing the individual capsaicinoid analogue concentrations to SHU rating demonstrated that the concentrations of capsaicin and dihydrocapsaicin were good predictors of the pungency of the oleoresin capsicum sample (Fig. 3B). For example, oleoresins having an SHU rating $\leq 5.0 \times 10^5$ SHU contained approximately 65% capsaicin and dihydrocapsaicin and samples with an SHU rating $\geq 1.0 \times 10^6$ consisted of >80% capsaicin and dihydrocapsaicin.

Since both fresh peppers and oleoresin capsicum exhibited variability in the concentrations of the capsaicinoids, we were not surprised find significant differences in the total and relative concentrations of capsaicinoids of the pepper sprays. An interesting discovery was that variability was present in all commercially available pepper sprays even in samples from the same manufacturer (Table 3). For example, two identical pepper spray products from different lots and the same manufacturer exhibited striking differences in total capsaicinoid concentration, 2.5 ± 0.2 μg/μL versus 13.5 ± 0.1 μg/μL. Variability in product composition was also observed in products from different manufacturers that were both labeled as 10% oleoresin capsicum with a rating of 2.0×10^6

SHU. The two products had vastly different capsaicinoid concentrations (0.95 ± 0.03 μg/μL versus 16.0 ± 0.4 μg/μL). Estimating the SHU value based on the total capsaicinoid concentration of the pepper sprays suggested that the labeled SHU values were sometimes overstated by a factor of >100 times (see Table 4).

Trends suggesting that the relative analogue concentrations of capsaicin and dihydrocapsaicin were responsible for the SHU rating of the product were apparent for both fresh peppers and oleoresin capsicum samples. In pepper sprays, the relative concentrations of capsaicin and dihydrocapsaicin were >80%. This may imply that the majority of the pepper sprays may have been prepared as dilutions of oleoresin capsicum having an SHU rating $\geq 1.0 \times 10^6$. Unfortunately, dilution of oleoresin capsicums having similar SHU rating does not appear to guarantee consistency in the amount of active ingredient in the pepper spray. Therefore, the variability in the capsaicinoid concentrations of the pepper sprays can be attributed to the inherent variability in the capsaicinoid concentrations of different batches of oleoresin capsicum and ultimately the particular properties of the extracted peppers.

From our data, it is reasonable to conclude that manufacturers of oleoresin capsicum-based pepper sprays do not standardize their product for the concentration of capsaicinoids. This was not true for samples PS8 and PS9 (Table 4). The manufacturer of this product used a known quantity of nonivamide as the active ingredient rather than a standard volume of oleoresin capsicum. Previous work has demonstrated that nonivamide exhibits identical pungency to capsaicin based upon the Scoville Organoleptic Test (12,13,18). It also has a similar structure activity relationship using desensitization of the VR receptor as a model system to predict pungency (12–18). Therefore, products fortified with or composed solely of nonivamide are likely to have similar efficacy to oleoresin capsicum-based pepper sprays for use as self-defense weapons. However, the studies reported here did not evaluate or compare the biological efficacy of products.

Nonivamide is not commonly used to manufacture pepper sprays because it is considered a "synthetic" and/or pharmaceutical agent. Oleoresin capsicum is a natural product. However, pepper sprays manufactured from oleoresin capsicum are not regulated. Thus far, the identification of nonivamide in fresh peppers has not been definitively confirmed and its existence as a natural product debated (3). Some authors suggested that nonivamide did not occur naturally because of the structure of its acyl chain (3). This argument was supported by inconclusive analytical data (3). GC/MS methods were ineffective at differentiating nordihydrocapsaicin and nonivamide since they have the same molecular weight (3,26–31). Standard HPLC methods did not chromatographically separate capsaicin from nonivamide (3, 31–35). The method described here allowed us to uniquely identify and quantify nonivamide in extracts of fresh pepper, oleoresin capsicum, and peppers sprays. Nonivamide was present at a concentration that represented up to 7.2% of the total capsaicinoid concentration (Tables 1–3).

Based on our work, several conclusions about the concentrations of capsaicinoids in peppers as well as products manufactured from extracts of peppers can be made. Because oleoresin capsicum is obtained by extracting fresh peppers, variability in the total and relative capsaicinoid concentrations exists. As such, products made from the oleoresin capsicum (e.g., food products, pepper sprays, etc.) also exhibit variability. Differences in the concentrations of active components in pepper sprays may affect the quality, efficacy, and safety of these products (20,37,38). Discrepancies in the amount of active ingredient in the products may result in unpredictable results when the products are used for self-defense or to subdue suspects. Differences in the concentration of active ingredients of the products could also affect the potential toxicity of the products and could jeopardize the safety and health of individuals who are exposed to and/or depend upon the products for protection (20,37–39). Quantitative analytical methods are needed to determine the concentration of capsaicinoids in pepper sprays. Implementing this objective quality control measure, as well as regulating the formulation of pepper sprays, would substantially increase the predictability of product potency, efficacy, and its potential to cause toxicity.

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